New *in vitro* Gastrointestinal Model Accurately Predicts Arsenic Bioavailability in Soils

Valerie L. Mitchell[1]; Shane Whitacre[2]; Stan W. Casteel[3]; Perry A. Myers[1]; Nicholas T. Basta[2]

(1) Department of Toxic Substances Control, Cal EPA, (2) Ohio State University, Columbus (3) University of Missouri, Columbia

ABSTRACT

Arsenic (As) is a naturally occurring metalloid commonly found in soil and a key chemical of concern at many brownfield sites. Risk assessment calculations typically utilize default oral toxicity values, which are based on ingestion of readily soluble forms of As dissolved in water. Arsenic in soils, however, is bound to various other minerals that result in decreased solubility/bioavailability of As. Historically, the use of the juvenile swine method was the only available method for determining the relative bioavailability (RBA) of As in soils. The EPA recently released guidance recommending a default bioavailability of 60% for arsenic in soils. It has been demonstrated, however, that the RBA of arsenic in soils can be as low as 1%. *In vitro* methodologies have proven to be useful surrogates for *in vivo* feeding studies in predicting RBA for other metals but lack precision for arsenic. The purpose of this study is to develop a single extraction *in vitro* procedure that conservatively estimates *in vivo* RBA of As for every test soil. Study soils were collected from mining sites throughout California with As ranging from 200 to 12,000 mg/kg and RBAs ranging from 1-40%. A modification of a previously published method (OSU-IVG) conservatively predicted As RBA in all study soils (n=18) and for most soils (9/11) with <1,200 mg/kg As the method provides a good estimate (within 90% CI) of RBA. This result holds true when ten non-California soils with swine RBA are included. The combined dataset provides enough data points for a robust regression (RBA= Modified OSU-IVG(0.8)+4.39, r^2=0.82). In summary, we have developed a new method for predicting bioavailability of As in soils. While the data is still preliminary in nature, this affordable bench-top method could be used in place of the more expensive juvenile swine *in vivo* studies to estimate RBA of As in soils. This data can then be used to adjust human health risk assessment equations and provide a more reasonable estimation of risk.

Sample Collection

Phase I Sampling

25 Soil Samples Collected from Empire Mine ("EM") State Historic Park and Rattlesnake Gates ("RG")

15 to 12,095 mg/kg Arsenic
Relative Bioavailability (n=12): 4-24%

Phase II Sampling ("New CA Soils")

14 Soils Samples Collected from Mining Sites in 5 Counties Throughout California

234 to 5598 mg/kg Arsenic
Relative Bioavailability (n=6): 1-40%

Other Samples Used in the Regression (SERDP, "SE")

10 non-California Soils with various Arsenic Source Contributions: Pesticide, Mining, Smelters, and Orchards

64 to 840 mg/kg Arsenic
Relative Bioavailability (n=10):33-80 %

Existing Methods for Estimating Bioavailability of Arsenic

- ***In vivo* Relative Bioavailability (RBA)**
- **Gold Standard, EPA Approved**
- **Juvenile Swine, University of Missouri**
  - **Animals dosed daily for 14 days**
  - **Absorbed arsenic measured in excreted urine**
  - **Test Soils compared to Sodium Arsenate Control**
- **Cost Prohibitive, Time Consuming**

SBRC (EPA 9200)

- **1g Soil:100mL Solution**
- **GE: 0.4M glycine,**

pH 1.5, 1 hour

OSU-IVG

- **1g Soil:150mL Solution**
- **GE: 0.1M NaCl, 1%pepsin, pH 1.8, 1 hour**
- **IE: GE + bile & pancreatin, pH 6.5, 2 hours**

Soil ID	OSU GE RBA prediction Basta 2009	OSU IE RBA prediction Basta 2009	EPA 9200 SBRC prediction Juhasz 2009	RBA (90% CI)
	%	%	%	%
EM1	18.61	27.6	6.36	23.7 (10.9-36.5)
EM3	10.49	13.4	2.93	15.3 (11.7-18.8)
EM5	11.38	15.0	2.75	15.3 (15.22-15.5)
EM8	10.28	13.9	3.24	19.2 (16.9-21.4)
EM13	9.52	12.4	2.75	12.5 (5.1-19.9)
EM15	11.16	14.9	6.14	19.7 (13.1-26.2)
EM18	8.60	9.9	3.67	4.0 (3.3-4.6)
EM19	8.93	11.7	2.01	11.7 (8.3-15.2)
EM20	17.04	21.9	12.36	22.7 (21.1-24.3)
EM21	15.99	21.3	15.92	23.0 (17.6-28.5)
RG1	8.59	13.0	2.65	11.8 (6.9-16.6)
RG3	10.58	13.2	2.79	12.4 (7.6-17.2)

- **Yellow highlight indicates under prediction of RBA.**
- **OSU-IVG under predicts less due to intercept in regression equation.**

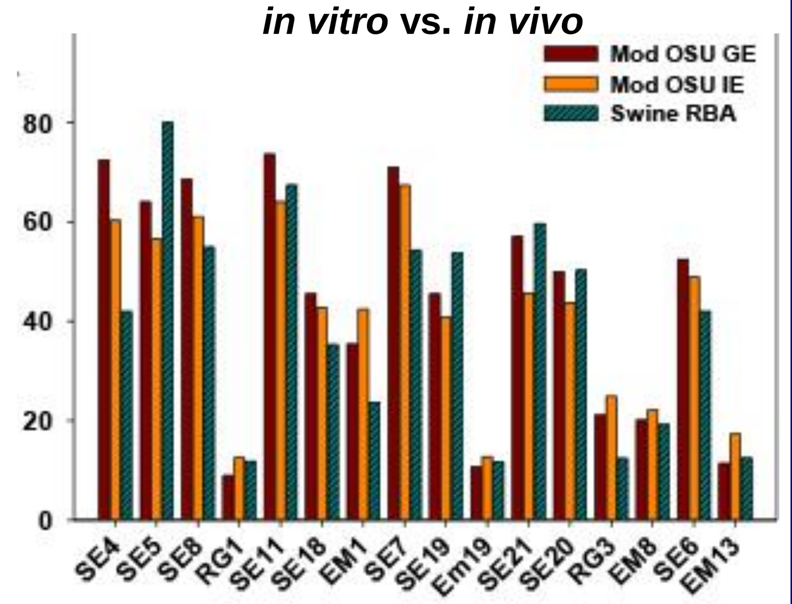
Modified OSU *in vitro* Assay as compared to *in vivo* Swine RBA

Modified OSU-IVG

- **1g Soil:150mL Solution**
- **GE: 0.1M NaCl, 1%pepsin, pH 1.5, ascorbic acid, 2 hour**
- **IE: GE + bile & pancreatin, pH 7.5, 4 hours**

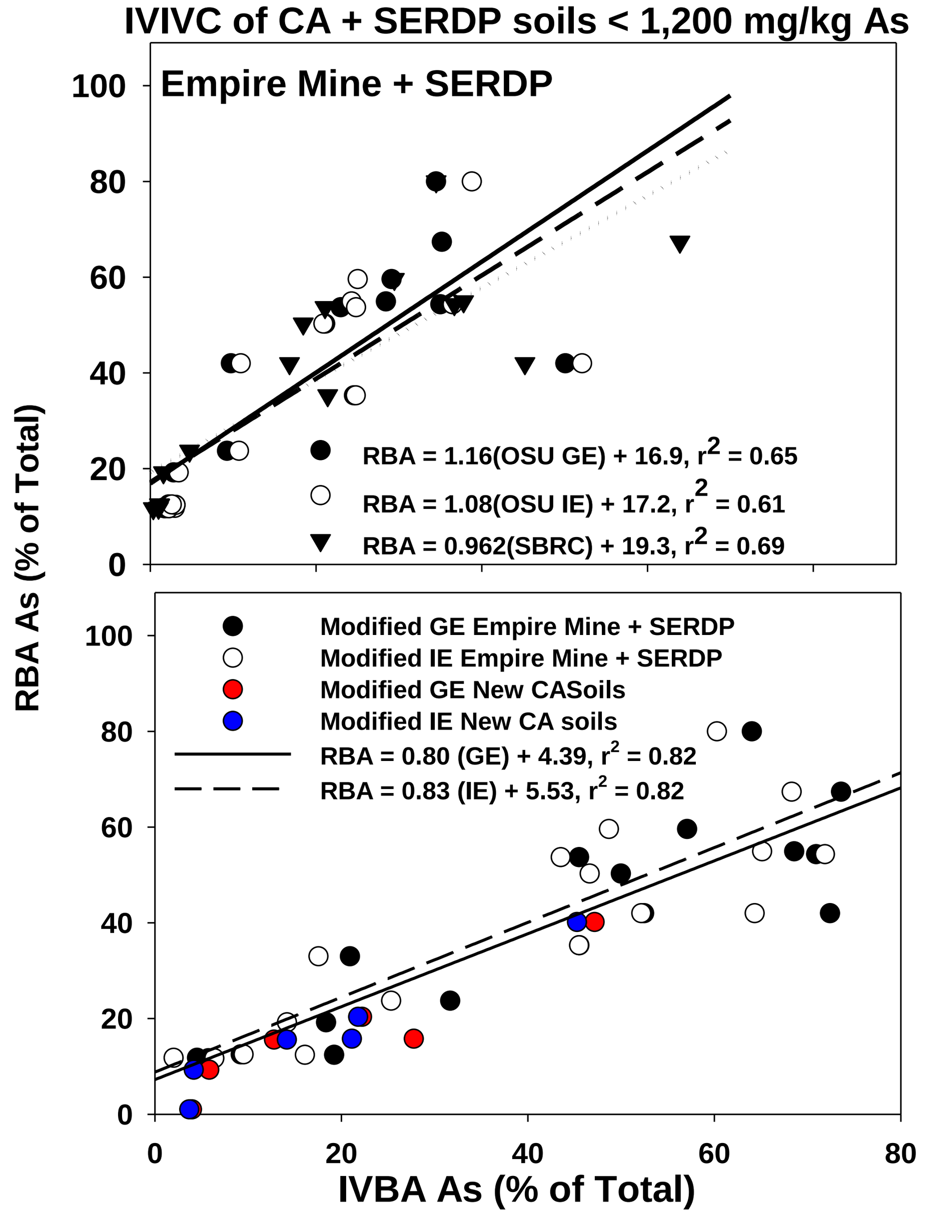
Percentage of Available Arsenic

in vitro* vs. *in vivo



Comparison of Regression Equations in New vs. Existing *in vitro* Methods

IVIVC of CA + SERDP soils < 1,200 mg/kg As



IVIVC: in vitro/in vivo comparison

<u>Guidelines for a Robust Regression:</u>

Slope between 0.8 and 1.2 i.e. *in vitro* ≈ *in vivo*

r^2 > 0.6

Intercept not deviate significantly from zero

Wragg et al. 2011, Sci. Total Environ.

When Should Bioavailability Adjustments Be Made?

Highly Contaminated

High level of total As
Bioavailability has to confidently be very low
Unreasonable adjustment

Moderately Contaminated

Moderate level:
up to 1000? mg/kg As
Reasonable adjustment

Summary of Findings

- **Current in vitro methods do not consistently measure or predict the bioavailable As in CA gold mine soils**
- **With the current CA and SERDP data set, Modified OSU-IVG could be used to make adjustments to site RBA by use of IVBA As as a conservative estimate of RBA or regression analysis to predict RBA.**
- **Use of IVBA As as a conservative estimate of RBA is a new approach, but does not have the problems associated with predictive equations from regression analysis.**

Next Steps

- **Validate Modified OSU *in vitro* Method in Independent Laboratories**
  - **Identify Outside Labs for Validation**
  - **Obtain US EPA Method Approval/ Certification**
- **Draft Guidance Document for Evaluating Arsenic for Site Cleanups**

References

1. Basta NT, et al, 2007. The effect of dosing vehicle on arsenic bioaccessibility in smelter-contaminated soils. J. Environ. Health Sci.
2. Juhasz AL, et al, 2009, Assessment of four commonly employed in vitro arsenic bioaccessibility assays for predicting in vivo relative arsenic bioavailability in contaminated Soils, Environ Sci Technol
3. US Environmental Protection Agency, 2012, EPA 9200.2-86, Standard Operation Procedure for an *In Vitro* Bioaccessibility Assay for Lead in Soil http://www.epa.gov/superfund/bioavailability/pdfs/EPA_Pb_IVBA_SOP_040412_FINAL_SRC.pdf
4. US Environmental Protection Agency, 2012, Recommendations for Default Value for Relative Bioavailability of Arsenic in Soil http://www.epa.gov/superfund/bioavailability/pdfs/Arsenic%20Bioavailability%20POLICY%20Memorandum%2012-20-12.pdf
5. Whitacre SD 2009. M.S. Thesis. Soil controls on arsenic bioaccessibility: Arsenic fractions and soil properties. The Ohio State University, Columbus, OH.
6. Wragg J, et al, 2011, An inter-laboratory trial of the unified BARGE bioaccessibility method for arsenic, cadmium and lead in soil, Sci Total Environ.

Funded by USEPA Brownfields Training, Research and Technical Assistance Grant TR-83415101